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Brief Report

*Editorial by Joshua J. Meeks on pp. 121–122 of this issue*

# A Novel Heterologous Prime-boost Strategy Using RUTI Vaccine to Improve the Bacillus Calmette-Guérin Response in Non–muscle-invasive Bladder Cancer Patients: Phase 1 RUTIVAC-1 Trial

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**Abstract**

Intravesical bacillus Calmette-Guérin (BCG) is the standard treatment for high-risk non–muscle-invasive bladder cancer (NMIBC); however, recurrence and progression remain major challenges. To explore a heterologous prime-boost strategy, we conducted the RUTIVAC-1 trial (NCT03191578) to evaluate RUTI, a nonlive vaccine, derived from *Mycobacterium tuberculosis*, as a systemic primer prior to intravesical BCG in high-risk NMIBC patients. The immunogenicity, safety, and preliminary efficacy of RUTI was evaluated. In this phase 1, randomized, double-blind, placebo-controlled trial, 40 NMIBC patients were randomized 1:1 to receive two subcutaneous doses of RUTI (25 μg) or placebo before BCG treatment. Immunogenicity was assessed by flow cytometry, and clinical follow-up was extended to 5 yr. RUTI induced a systemic vaccine-specific response, increasing activation markers on $CD4^+$ and $CD8^+$ T cells, supporting effective immune priming. Regulatory T-cell expansion was observed in the placebo group but not in RUTI-vaccinated patients, who also exhibited a more heterogeneous and polyfunctional specific immune response. RUTI was safe and well tolerated, with only mild injection-site reactions. Exploratory analyses showed a trend toward reduced recurrence, progression, and death, with improved 5-yr progression-free survival. The small sample size and baseline imbalances were the limitations. This phase 1 study supports the safety and

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immunogenicity of RUTI and provides encouraging data that warrant confirmation in larger trials to establish its potential clinical benefit as a novel therapeutic strategy.



**ADVANCING PRACTICE**

**What does this study add?**

This study demonstrates that RUTI vaccination before intravesical bacillus Calmette-Guérin (BCG) therapy in patients with non–muscle-invasive bladder cancer is safe and well tolerated, and enhances BCG-induced immunity by promoting a more diverse and polyfunctional T-cell response. Regulatory T-cell expansion was observed in the placebo group but not in RUTI-vaccinated patients, which may improve therapeutic efficacy. Preliminary efficacy results, supported by a subanalysis in high-grade T1 patients, suggest that RUTI vaccination could improve survival outcomes. These encouraging findings warrant further investigation in larger phase 2b/3 trials to confirm its clinical benefit.

**Clinical Relevance**

In a randomized, double-blind, placebo-controlled phase 1 trial, two subcutaneous priming doses of the nonlive mycobacterial vaccine RUTI administered before induction BCG were feasible and well tolerated (primarily mild injection-site reactions). Exploratory 5-year analyses suggested lower recurrence and higher progression-free survival with RUTI versus placebo (estimated 100% vs 63.3% overall; 100% vs 72.2% in a T1 high-grade/CIS-negative subgroup), acknowledging the small sample size and baseline imbalances. Clinically, these data support a practical, low-toxicity prime-boost strategy that may enhance BCG responsiveness and justify phase 2b/3 confirmation to determine whether pre-BCG vaccination should be integrated into standard care. Associate Editor: Laura Bukavina, MD.

**Patient Summary**

In this study, we tested a new vaccine called RUTI, given before standard intravesical bacillus Calmette-Guérin (BCG) treatment in patients with non–muscle-invasive bladder cancer. The vaccine was safe and triggered a specific immune response that may help the body respond more effectively to subsequent BCG therapy. Exploratory analyses showed a trend toward reduced recurrence, progression, and death after RUTI vaccination. These encouraging findings require further confirmation in larger studies to validate the potential of RUTI as a new strategy to improve treatment outcomes for patients with non–muscle-invasive bladder cancer.

Bladder cancer is among the most prevalent urologic malignancies [1], with non–muscle-invasive bladder cancer (NMIBC) accounting for >75% of new diagnoses. Despite intravesical bacillus Calmette-Guérin (BCG) being the standard of care for high-risk NMIBC, recurrence and progression rates remain substantially high [2]. Therefore, strategies to enhance the therapeutic efficacy of BCG without increasing toxicity represent a critical unmet clinical need.

RUTI, a safe, nonlive vaccine originally designed for tuberculosis, contains detoxified liposomal cell wall fragments of *Mycobacterium tuberculosis* and induces a Th1-polarized immune response [3,4]. Given the antigenic overlap with BCG (*Mycobacterium bovis*), we hypothesized that RUTI priming prior to intravesical BCG could enhance BCG-induced immunity in NMIBC patients. To test this, we conducted a randomized, double-blind, placebo-controlled, phase 1 study (RUTIVAC-1; NCT03191578) to evaluate the immunomodulatory effects of this heterologous prime-boost strategy (clinical trial details are included in the Supplementary material).

In this pilot study, 40 patients were randomized to receive two subcutaneous doses of RUTI (25 μg) or placebo prior to intravesical BCG (Supplementary Fig. 1). The primary endpoint was the change in systemic antigen-specific immune response, evaluated by flow cytometry using activation-induced marker (AIM) and intracellular cytokine staining assays after ex vivo purified protein derivative (PPD) stimulation, along with phenotypic profiling of peripheral immune cells (Supplementary Fig. 2). Samples were collected at baseline and at weeks 2, 6, and 16. The secondary endpoints included clinical outcomes (recurrence, progression, and death) and safety (Supplementary material).

Baseline demographics were comparable between groups; however, tumor-related characteristics were imbalanced despite double-blind randomization (Table 1), likely due to the small sample size. High-grade T1 tumors and multifocal lesions were more frequent in the placebo group (75% vs 55% and 63% vs 26%, respectively). Carcinoma in situ (CIS) was present only in the placebo group (4%). Tumor size was similarly distributed. Most patients completed BCG

**Table 1 – Patient demographic and tumor characteristics at baseline**

| | Vaccine administered | |
|---|---|---|
| | RUTI (*N* = 20) | Placebo (*N* = 20) |
| Age (yr), median (IQR) | 71 (62–77) | 67 (59–74) |
| Gender (male), *n* (%) | 17 (85) | 19 (95) |
| Weight (kg), median (IQR) | 77 (70–84) | 69 (66–81) |
| Body mass index (kg/m$^2$), median (IQR) | 27.2 (25–30) | 26 (23–28) |
| Smokers, *n* (%) | 14 (70) | 19 (95) |
| Cancer stages and grades, *n* (%) | | |
| Ta HG | 9 (45) | 4 (20) |
| T1 LG | 0 (0) | 1 (5) |
| T1 HG | 11(55) | 15 (75) |
| Presence of concomitant CIS, *n* (%) | 0 (0) | 4 (20) |
| Ta + CIS | 0 (0) | 1 (5) |
| T1 + CIS | 0 (0) | 3 (15) |
| No. of lesions, *n* (%) | | |
| Solitary | 14 (73.7) | 7 (36.8) |
| Multiple | 5 (26.3) | 12 (63.1) |
| Not documented | 1 (5) | 1 (5) |
| Tumor size, *n* (%) | | |
| Small (≤1–3 cm) | 10 (58.8) | 9 (53) |
| Large (>3 cm) | 7 (41.2) | 8 (47) |
| Not documented | 3 (15) | 3 (15) |
| BCG treatment total instillations [a], *n* (%) | | |
| <15 instillations | 3 (15) | 3 (15) |
| 15 instillations | 14 (70) | 17 (85) |
| >15 instillations | 3 (15) | 0 (0) |

BCG = bacillus Calmette-Guérin; CIS = carcinoma in situ; EAU = European Association of Urology; IQR = interquartile range; LG = low grade; HG = high grade.

[a] BCG treatment total instillation was defined as at least 15 instillations (six instillations in the induction course and three instillations at 3, 6, and 12 mo in the maintenance course; according to the EAU guidelines) [2]. The total number of instillations per patient is shown in Fig. 1A.

treatment. To address this imbalance, an exploratory subgroup analysis was conducted in high-grade T1 patients without CIS, matched for other tumor features (clinical course and treatment response are shown in Fig. 1A and Supplementary Table 1).

To first assess the immunogenicity of the vaccine, we evaluated antigen-specific responses following PPD stimulation. RUTI effectively primed a systemic antigen-specific immune response, increasing AIMs (CD25, CD69, CD137, and OX40) on CD4$^+$ and CD8$^+$ T cells, with no changes in the placebo group (Fig. 1B and Supplementary Fig. 3A). CD4$^+$ activation was mainly in CD27$^-$ cells, while CD8$^+$ activation occurred in both CD27$^+$ and CD27$^-$ subsets (Supplementary Fig. 3B and 3C). RUTI also increased dual AIM$^+$ T cells (Supplementary Fig. 3D), supporting systemic immune priming prior to the initiation of intravesical BCG therapy.

In the placebo group, a transient increase in CD4$^+$ T cells was observed after intravesical BCG, but not seen in RUTI-vaccinated patients (Supplementary Fig. 4A). Notably, in the placebo group, a significant increase was observed in CD4$^+$ expressing CD25$^+$ but lacking other AIMs and phenotypically consistent with T regulatory cells (Treg cells: CD4$^+$CD25$^+$CD27$^+$; Fig. 1C and Supplementary Fig. 4B and C). This increase in Treg cells was not seen in the RUTI group.

Following PPD stimulation, the placebo group showed a skewed CD4$^+$CD25$^+$CD27$^+$ profile, whereas RUTI-treated patients displayed a more heterogeneous CD4$^+$ AIM$^+$ response (CD25$^+$, CD137$^+$, OX40$^+$, and CD69$^+$), predominantly in CD27$^-$ cells, with some subsets remaining elevated at W16 (Supplementary Fig. 5). Moreover, higher PPD-specific cytokine responses were observed in the RUTI group, including increased frequencies of IFN-$\gamma^+$ and IL-2$^+$, total cytokine-expressing CD4$^+$ T cells, and a higher frequency of polyfunctional CD4$^+$ T cells expressing multiple effector cytokines (Fig. 1D and E, and Supplementary Fig. 5D). To evaluate RUTI-specific responses, *M. tuberculosis* antigens absent in BCG (ESAT-6 and HSP16.3) were used. Increased cytokine-producing CD4$^+$ and CD8$^+$ T cells were detected following stimulation, indicating a vaccine-specific response occurring concomitantly with intravesical BCG treatment (Supplementary Fig. 6).

Although efficacy outcomes were exploratory due to small size and tumor imbalance, RUTI-treated patients showed a trend toward lower recurrence, progression, and death (Fig. 1A and F, and Supplementary Fig. 7A). This difference was maintained in the T1 high-grade or CIS-negative subgroup (Fig. 1G and Supplementary Fig. 7B), with 5-yr estimated progression-free survival (PFS) rates of 100% (RUTI) versus 63.3% (placebo) and 100% versus 72.2%, respectively, in the full cohort. RUTI vaccine was safe and well tolerated, with only mild injection-site reactions reported (Supplementary Table 2).

This pilot phase 1 trial is the first to evaluate a heterologous prime-boost strategy combining RUTI vaccination with intravesical BCG in high-risk NMIBC patients. RUTI was safe and well tolerated, and enhances systemic BCG-specific immune responses.

Previous studies suggest that pre-existing BCG-specific T cells by prior mycobacterial exposure improve BCG efficacy [5,6]. In our study, RUTI effectively primed a systemic specific immune response, increasing heterogeneous PPD-specific CD4$^+$ and CD8$^+$ T cells prior to intravesical BCG. In addition, RUTI-treated patients displayed a broader, more polyfunctional immune profile and reduced Treg expansion, an immunosuppressive effect typically associated with BCG [7].These findings align with prior tuberculosis data suggesting that mycobacterial immunity can be enhanced by limiting Treg responses [8]. Our study shows that while CD8$^+$ T cells contributed to RUTI + BCG treatment response, CD4$^+$ T cells play a central role, confirming their key role in antitumor immunity [9]. However, our immune analysis was limited, and the involvement of other BCG-responsive populations, such as NK, γδ T cells, or B cells, cannot be ruled out [6,10].

Regarding efficacy, although exploratory, our findings, supported by a subanalysis in high-grade T1 patients, suggest that RUTI vaccination may enhance BCG responses and improve survival outcomes.

The analysis is limited by the small sample size, baseline imbalance, and absence of bladder immune infiltration data. Additionally, the lack of a second transurethral resection of the bladder before BCG may have contributed to early recurrences and progression events, although all patients had negative cystoscopy and cytology before inclusion.

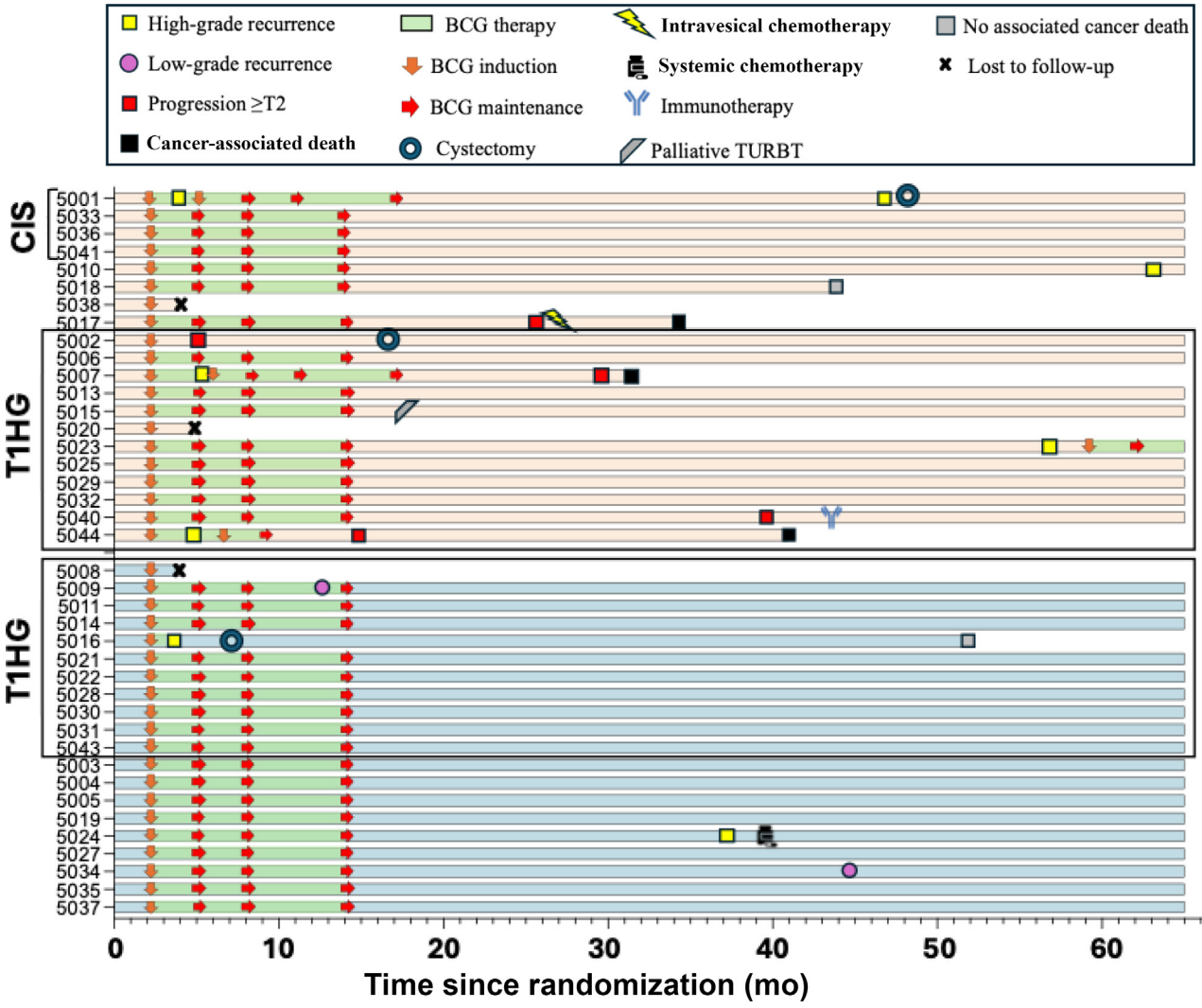


**Fig. 1 – Clinical course and systemic immunomodulation by RUTI vaccination in the RUTIVAC-1 trial. (A) Swimmer plot illustrating individual patient follow-up in months from randomization, including the timing of clinical events (recurrence, progression, additional treatment, death, and loss to follow-up). Patients with concomitant carcinoma in situ (CIS) and high-grade T1 (T1HG) are indicated. Placebo-vaccinated patients are shown in orange and RUTI-vaccinated patients in blue; dots represent individual patients. (B) Modulation of the immune response by RUTI administration before BCG intravesical treatment. Fold change values in activation-induced marker expression in $CD4^+$ T cells at week 2 (after two vaccine doses) versus baseline (BL) following ex vivo PPD stimulation. (C) Longitudinal changes in $CD4^+CD25^+$ and $CD4^+CD25^+CD27^+$ T cells at weeks 6 and 16 versus BL in the in the absence of ex vivo stimulation. Cytokine-based PPD-specific $CD4^+$ T-cell responses over time: frequency of (D) total cytokine-expressing $CD4^+$ T cells and (E) polyfunctional $CD4^+$ T cells producing two or three cytokines. Kaplan-Meier curves showing the 5-yr follow-up of placebo- and RUTI-vaccinated patients for (F) the overall study population and (G) the T1HG cohort. High-grade recurrence-free survival (RFS) considering only high-grade recurrences and progression-free survival (PFS) are presented for patients who received either placebo or RUTI vaccine. Hazard ratios and 95% confidence intervals are also provided. Statistical significance was assessed using the log-rank test. (B, C) The data are displayed as box-and-whisker plots (5–95th percentile). Statistically significant differences were calculated using a Wilcoxon signed-rank nonparametric test (within-group fold change vs 1); Wilcoxon matched-pair signed-rank nonparametric test (paired samples at W6 vs W16; black lines), and Mann-Whitney *U* nonparametric test (between-group comparisons; blue lines; $^{\#}$ $p \le 0.05$; $^{\#\#}$ $p \le 0.01$). (D, E) Bars represent mean ± standard error of the mean. The *p* values were calculated using paired two-tailed Wilcoxon tests for comparisons between time points in the same individual (black lines). BCG = bacillus Calmette-Guérin; PPD = purified protein derivative; TURBT = transurethral resection of a bladder tumor; W = week. $^{*}$ $p \le 0.05$. $^{**}$ $p \le 0.01$. $^{***}$ $p \le 0.001$.**

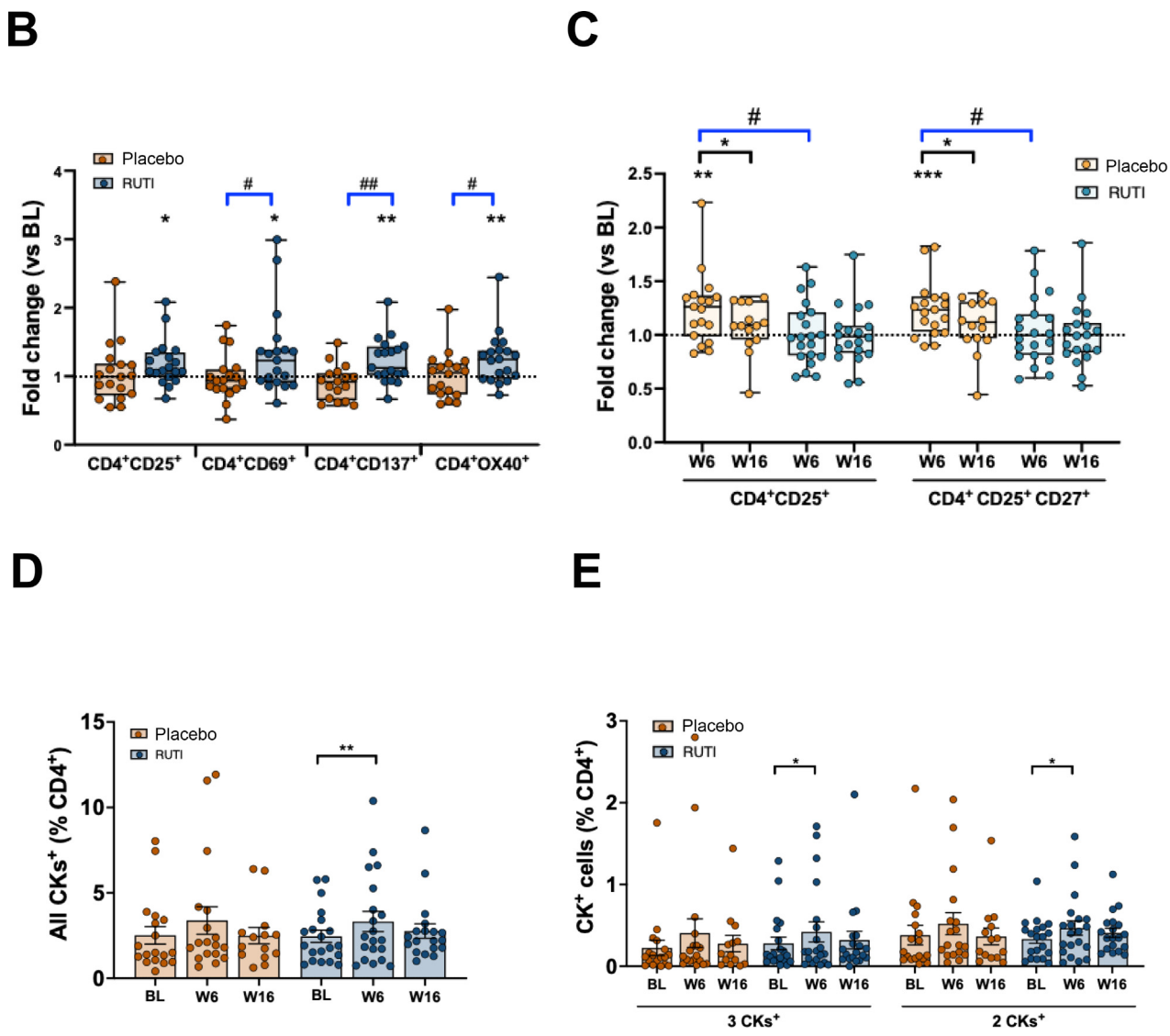


**Fig. 1** (*continued*)

In conclusion, this study suggests that RUTI modulates the BCG-induced immunity by promoting a more diverse, polyfunctional, and balanced effector/regulatory response. This phase 1 study supports the safety and immunogenicity of RUTI in NMIBC patients receiving intravesical BCG, providing encouraging data that warrant confirmation in larger phase 2b trials to stablish its potential clinical benefit as a novel therapeutic strategy.

***Author contributions*:** Cecilia Cabrera had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

*Study concept and design*: Cabrera, Buisan, Servian, Martínez, Cardona, Vilaplana.
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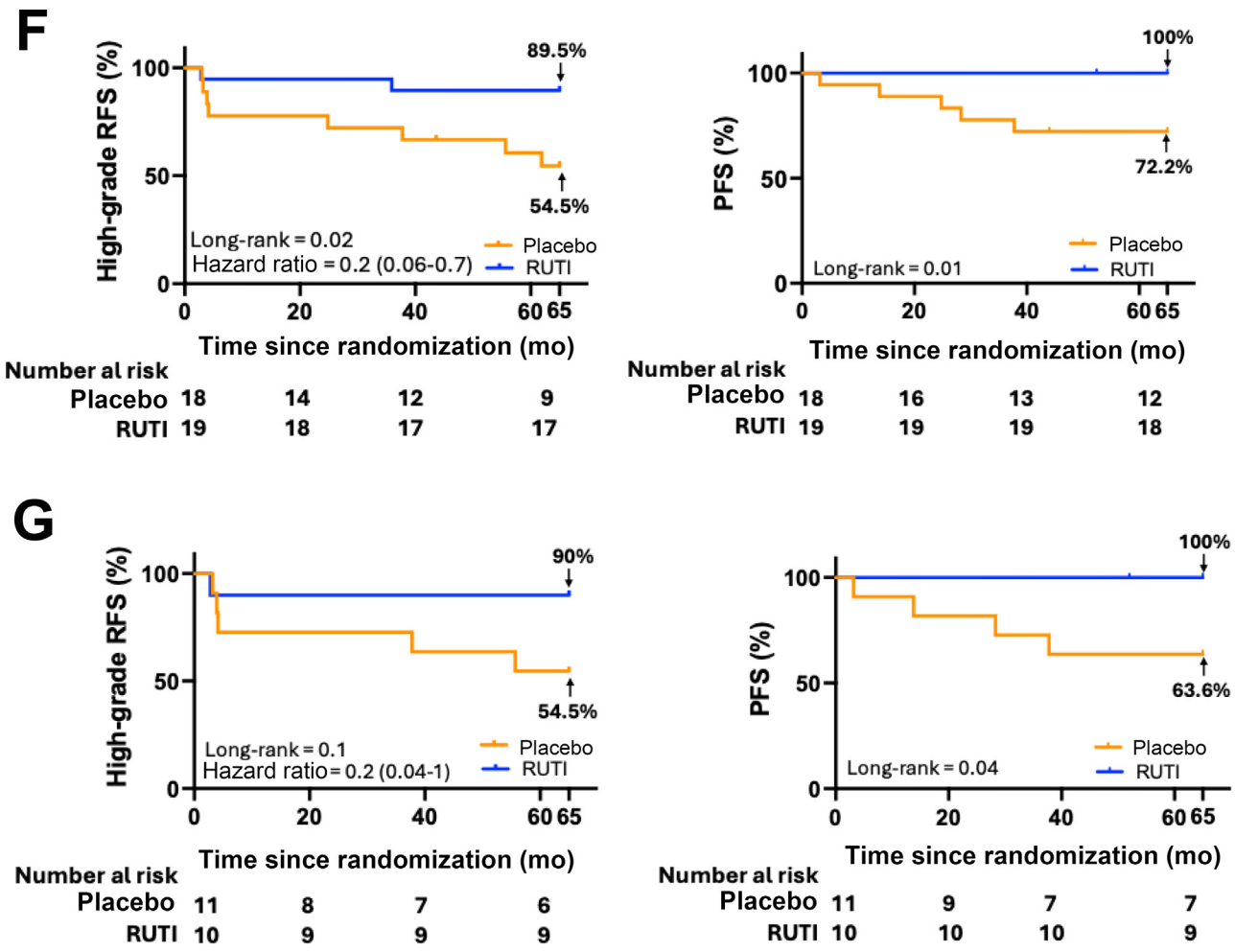


**Fig. 1** (*continued*)

bladder cancer using RUTI vaccination. This is based on the results of this work. Joaquim Bellmunt has served in consulting or advisory roles for Astellas Pharma, AstraZeneca/MedImmune, Bristol Myers Squibb, Genentech, Novartis, Pfizer, Pierre Fabre, and the healthcare business of Merck KGaA, Darmstadt, Germany; has received travel and accommodation expenses from Ipsen, Merck & Co., Kenilworth, NJ, USA and Pfizer; reports patents, royalties, and other intellectual property from UpToDate; reports stock and other ownership interests in Rainier Therapeutics; has received honoraria from UpToDate; and has received institutional research funding from Millennium, Pfizer, Sanofi, and the healthcare business of Merck KGaA, Darmstadt, Germany. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eururo.2025.08.014.

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